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(71) Applicants *(for all designated States except US)*: SMITHKLINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US). SMITHKLINE BEECHAM PLC [GB/GB]; Three New Horizons Court, Great West Road, Brentford, Middlesex TW8 9EP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LEVY, Mark, Alan [US/US]; 115 reveille Road, Wayne, PA 19087 (US). LEE, Dennis [CA/US]; 205 Haverford Avenue, Swarthmore, PA 19081 (US). GLEASON, John, Gerald [CA/US]; 8 Heron Hill Drive, Downington, PA 19335 (US). TAYLOR, Andrew, William [GB/GB]; 64 Mazoe Road, Bishops Stortford CM23 3JT (GB). CORBERTT, David, Francis [GB/GB]; 12 Wilmots Close, Reigate, Surrey RH2 0NP (GB).

(74) Agents: VENETIANER, Stephen et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

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(57) Abstract

The present invention is to the novel compounds of Formula (I), their pharmaceutical compositions, and to the novel inhibition of ICE and ICE-like proteins for use in the treatment of apoptosis, and disease states caused by excessive or inappropriate cell death.

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Interleukin Converting Enzyme and Apoptosis

FIELD OF THE INVENTION

The present invention is to the discovery of a new method to block excessive or inappropriate apoptosis in a mammal.

BACKGROUND

It has been recognized for over a century that there are different forms of cell death. One form of cell death, necrosis, is usually the result of severe trauma and is a process that involves loss of membrane integrity and uncontrolled release of cellular contents, often giving rise to inflammatory responses. In contrast, apoptosis is a more physiological process that occurs in a controlled manner and is generally non-inflammatory in nature. For this reason apoptosis is often referred to as programmed cell death. The name itself (apoptosis: Greek for "dropping off", for example leaves from trees) implies a cell death that is part of a normal physiological process (Kerr et al., Br. J. Cancer, 26: 239-257 (1972)).

Apoptosis appears to be a carefully controlled series of cellular events which ultimately leads to death of the cell. This process for elimination of unwanted cells is active and requires expenditure of cellular energy. The morphological characteristics of apoptosis include cell shrinkage and loss of cell-cell contact, condensation of nuclear chromatin followed by fragmentation, the appearance of membrane ruffling, membrane blebbing and apoptotic bodies. At the end of the process, neighboring cells and macrophages phagocytose the fragments from the apoptotic cell. The process can be very fast, occurring in as little as a few hours (Bright et al., Biosci. Rep., 14: 67-82 (1994)).

The best defined biochemical event of apoptosis involves the orderly destruction of nuclear DNA. Signals for apoptosis promote the activation of specific calcium- and magnesium-dependent endonucleoases that cleave the double stranded DNA at linker regions between nucleosomes. This results in production of DNA fragments that are multiples of 180-200 base pair fragments (Bergamaschi et al., Haematologica, 79: 86-93 (1994); Stewart, JNCI, 86: 1286-1296 (1994)). When examined by agarose gel electrophoresis, these multiple fragments form a ladder pattern that is characteristic for most cells undergoing apoptosis.

There are numerous stimuli that can signal cells to initiate or promote cellular apoptosis, and these can be different in different cells. These stimuli can include glucocorticoids, TNFa, growth factor deprivation, some viral proteins, radiation and anticancer drugs. Some of these stimuli can induce their signals through a variety of cell surface receptors, such as the TNF / nerve growth factor family of receptors, which include CD40 and Fas/Apo-1 (Bright et al., supra). Given this diversity in stimuli that cause apoptosis it has been difficult to map out the signal transduction pathways and molecular factors involved in apoptosis. However, there is evidence for specific molecules being involved in apoptosis.

The best evidence for specific molecules that are essential for apoptosis comes from the study of the nematode C. elegans. In this system, genes that appear to be required for induction of apoptosis are Ced-3 and Ced-4. These genes must function in the dying cells and, if either gene is inactivated by mutation, cell death fails to occur (Yuan et al., Devel. Biol., 138: 33-41 (1990)). In mammals, genes that have been linked with induction of apoptosis include the proto-oncogene c-myc and the tumor suppresser gene p53 (Bright et al., supra; Symonds et al., Cell, 78: 703-711 (1994)).

In this critical determination of whether or not to undergo apoptosis, it is not surprising that these are genes that program for proteins that inhibit apoptosis. An example in C. elegans is Ced-9. When it is abnormally activated, cells survive that would normally die and, conversely, when Ced-9 is inactivated cells die that would normally live (Stewart, B.W., supra). A mammalian counterpart is bcl-2, which had been identified as a cancer-causing oncogene. This gene inhibits apoptosis when its product is overexpressed in a variety of mammalian cells, rendering them less sensitive to radiation, cytotoxic drugs and apoptotic signals such as c-myc (Bright et al., supra). Some viral proteins have taken advantage of this ability of specific proteins to block apoptosis by producing homologous viral proteins with analogous functions. An example of such a situation is a protein produced by the Epstein Barr virus that is similar to bcl-2, which prevents cell death and thus enhances viral production (Wells et al., J. Reprod. Fertil., 101: 385-391 (1994)). In contrast, some proteins may bind to and inhibit the function of bcl-2 protein, an example being the protein bax (Stewart, B.W., supra). The overall picture that has developed is that entry into apoptosis is regulated by a careful balancing act between specific gene products that promote or inhibit apoptosis (Barinaga, Science, 263: 754-756 (1994).

Apoptosis is an important part of normal physiology. The two most often cited examples of this are fetal development and immune cell development. In development of the fetal nervous system, over half of the neurons that exist in the early fetus are lost by apoptosis during development to form the mature brain (Bergamaschi et al., Haematologica, 79: 86-93 (1994)). In the production of immune competent T cells (and

to a lesser extent evidence exists for B cells), a selection process occurs that eliminates cells that recognize and react against self. This selection process is thought to occur in an apoptotic manner within areas of immune cell maturation (Williams, G. T., J. Pathol., 173: 1-4 (1994); Krammer et al., Curr. Opin. Immunol., 6: 279-289 (1994)).

Dysregulation of apoptosis can play an important role in disease states, and diseases can be caused by both excessive or too little apoptosis occurring. An example of diseases associated with too little apoptosis would be certain cancers. There is a follicular B-cell lymphoma associated with an aberrant expression of functional bcl-2 and an inhibition of apoptosis in that cell (Bergamaschi et al., supra). There are numerous reports that associate deletion or mutation of p53 with the inhibition of apoptosis and the production of cancerous cells (Kerr et al., Cancer, 73: 2013-2026 (1994); Ashwell et al., Immunol. Today, 15: 147-151, (1994)). In contrast, one example of excessive or inappropriate apoptosis is the loss of neuronal cells that occurs in Alzheimer disease, possible induced by b-amyloid peptides (Barr et al., BioTechnology, 12: 487-493 (1994)). Other examples include excessive apoptosis of $CD4^+$ T cells that occurs in HIV infection, of cardiac myocytes during infarction / reperfusion and of neuronal cells during ischemia (Bergamaschi et al., supra); Barr et al., supra).

Some pharmacological agents attempt to counteract the lack of apoptosis that is observed in cancers. Examples include topoisomerase II inhibitors, such as the epipodophyllotoxins, and antimetabolites, such as ara-c, which have been reported to enhance apoptosis in cancer cells (Ashwell et al., supra). In many cases with these anti-cancer drugs, the exact mechanism for the induction of apoptosis remains to be elucidated.

In the last few years, evidence has built that ICE and proteins homologous to ICE play a key role in apoptosis. This area of research has been spurred by the observation of homology between the protein coded by Ced-3, a gene known to be critical for C. Elegans apoptosis, and ICE. These two proteins share 29% amino acid identity, and complete identity in the 5 amino acid portion thought to be responsible for protease activity (QACRG) (Yuan et al., Cell, 75: 641-652 (1993)). Additional homologies are observed between ICE and the product of the nedd-2 gene in mice, a gene suspected of involvement in apoptosis in the developing brain (Kumar et al., Genes Dev., 8: 1613-1626 (1994)) and Ich-1 and CPP32 (ICE and Ced-3 homolog-1), human counterparts of nedd-2 isolated from human brain cDNA libraries (Wang et al., Cell, 78: 739-750 (1994); Fernandes-Alnemiri et al., J. Biol. Chem., 269: 30761-30764 (1994)).

Further proof for the role of these proteins in apoptosis comes from transfection studies. Over expression of murine ICE caused fibroblasts to undergo programmed cell

death in a transient transfection assay (Miura et al., Cell, 75: 653-660 (1993)). Cell death could be prevented by point mutations in the transfected gene in the region of greatest homology between ICE and Ced-3. As very strong support for the role of ICE in apoptosis, the authors showed that ICE transfection-induced apoptosis could be antagonized by overexpression of bcl-2, the mammalian oncogene that can prevent programmed cell death (Miura et al., supra). Additional experiments were performed using the crmA gene. This gene of the cowpox virus encodes a serpin protein, a family of proteins that are inhibitors of proteases (Ray et al., Cell, 69: 597-604 (1992)). Specifically, the protein of crmA has been shown to inhibit processing of pro-interleukin -1b by ICE. (Gagliardini et al. Science, 263: 826-828 (1994)) showed that microinjection of the crmA gene into dorsal root ganglion neurons prevented cell death induced by nerve growth factor deprivation. This result shows that ICE is involved in neuronal cell apoptosis. A more direct demonstration of ICE involvement comes from experiments in which ICE transfection is coupled with the co-expression of crmA, demonstrating a crmA-induced suppression of the ICE-induced apoptosis response (Miura et al., supra; Wang et al., supra).

In addition to ICE, researchers have examined the ability of ICE-like genes to promote apoptosis. (Kumar et al. supra) demonstrated that over expression of nedd-2 in fibroblasts and neuroblastoma cells resulted in cell death by apoptosis and that this apoptosis could also be suppressed by expression of the bcl-2 gene. Most recently, Wang et al., (Wang et al. , supra) examined the over expression of Ich-1 in a number of mammalian cells. Expression resulted in cell apoptosis, which could be antagonized by bcl-2 co-expression. Mutation of a cysteine residue, contained within the QACRG motif and presumed to be critical for protease function, to serine abolished apoptotic activity.

Further evidence for a role of a cysteine protease in apoptosis comes from a recent report by Lazebnik et al. (Nature, 371: 346-347 (1994)). These authors have used a cell-free system to mimic and study apoptosis. In their system there is a protease activity that cleaves the enzyme poly(ADP-ribose) polymerase at a site identical to a cleavage site in pre-interleukin-1b. However, this yet to be isolated protease and ICE appear to be different and to act on different substrate proteins. Blockade of protease activity in the system, using non-selective cysteine protease inhibitors, resulted in inhibition of apoptosis.

Taken together, the above evidence provides striking involvement of ICE and ICE-like proteins in the induction of apoptosis in mammalian cells. Brain interleukin-1 has been reported to be elevated in Alzheimer disease and Down syndrome (Griffin et al., Proc. Natl. Acad. Sci. U. S. A., 86: 7611-7615 (1989)). There are also reports that interleukin-1 can increase the mRNA and production of b-amyloid protein, a major

component of senile plaques in Alzheimer disease as well as in brains of people with Down syndrome and with aging (Forloni et al., Mol. Brain Res., 16: 128-134 (1992); Buxbaum et al., Proc. Natl. Acad. Sci. U. S. A., 89: 10075-10078 (1992); Goldgaber et al., Proc. Natl. Acad. Sci. U. S. A., 86: 7606-7610 (1989)). These reports can be viewed as additional evidence for the involvement of ICE in these diseases and the need for use of a novel therapeutic agent and therapy thereby.

To date, no useful therapeutic strategies have blocked excessive or inappropriate apoptosis. In one patent application, EPO 0 533 226 a novel peptide structure is diclosed which is said to be useful for determining the activity of ICE, and therefore useful in the diagnoses and monitoring of IL-1 mediated diseases. Therefore, a need exists to find better therapeutic agents which have non-toxic pharmacological and toxicological profiles for use in mammals. These compounds should block excessive or inappropriate apoptosis cells, and hence provide treatment for diseases and conditions in which this condition appears.

SUMMARY OF THE INVENTION

The present invention is to the novel compounds of Formula (I), their pharmaceutical compositions, and to the novel inhibition of ICE and ICE-like proteins for use in the treatment of apoptosis, and disease states caused by excessive or inappropriate cell death.

Another aspect of the present invention is to a pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

Another aspect of the present invention is to a method for the treatment of diseases or disorders associated with excessive IL-1b convertase activity, in a mammal in need thereof, which method comprises administering to said mammal an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

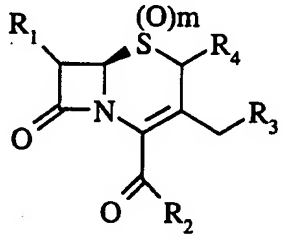
Another aspect of the present invention is to a method of preventing, or reducing apoptosis (i.e.blocking excess or inappropriate apoptosis) in a mammal, preferably a human, in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Another aspect of the present invention is to a method of blocking or decresing the production of IL-1b and/or TNF, in a mammal, preferably a human, in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the present invention may contain one or more asymmetric carbon atoms, in particular positions 6 and 7, and may exist in racemic and optically active forms. All of these compounds are included within the scope of the present invention. Preferably the compound has a 6R, 7S configuration.

Preferably the compounds of Formula (I) are represented by the structure:



(I)

wherein

R_1 is hydrogen, an optionally substituted alkoxy, or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, $-OC(O)R_5$, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl;

R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;

n is 0, or an integer having a value of 1 or 2;

or a pharmaceutically acceptable salt thereof.

Suitably, for compounds of Formula (I), R_1 is hydrogen, an optionally substituted C_{1-4} alkoxy or halogen. When R_1 is alkoxy, the carbon chain may be optionally substituted, one or more times, suitably one to three times, independently by hydroxy, halogen, alkoxy, C(O)H, $C(O)_2R_c$, or $C(O)CH_3$ moieties; wherein R_c is hydrogen, C_{1-6} alkyl, aryl, or arylC_{1-4}alkyl. Preferably R_1 is methoxy.

Suitably, for compounds of Formula (I), R_2 is OR_a; wherein R_a is C_{1-4}alkyl, or an optionally substituted arylC_{1-4}alkyl, preferably benzyl. It is recognized that the alkyl group in the arylalkyl moiety may be branched or straightsuch as a methylene or substituted methylene group , i.e., $-CH(CH_3)$ - aryl.

When R_a is an optionally substituted arylC_{1-4}alkyl, the aryl ring may be substituted one or more times independently by hydroxy, halogen, alkyl or alkoxy. When R_a is an alkyl, it is preferably methyl or t-butyl.

Suitably, for compounds of Formula (I), m is 1 or 2. Preferably m is 2.

Suitably, for compounds of Formula (I), R_3 is hydrogen, -OC(O)R_5, S(O)n-R_6, or bromo; provided that when R_3 is hydrogen, then R_4 is other than hydrogen. When R_3 is -OC(O)R_5, the R_5 group is suitably C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, or optionally substituted arylalkyl; preferably R_5 is C_{1-6} alkyl, more preferably methyl.

When R_3 is S(O)n R_6, R_6 is suitably an optionally substituted aryl, or an optionally substituted aryl heteroaryl; and n is 0, or an integer having a value of 1 or 2. When R_6 is heteroaryl, as defined below, it is preferably a triazole, oxadiazole, or tetrazole moiety. When R_6 is aryl, as also defined below, it is preferably a phenyl; the n value is preferably 1 or 2. When R_6 is a heteroaryl, n is preferably 0. The heteroaryl or aryl ring may be optionally substituted one or more times independently by hydroxy, halogen, alkyl or alkoxy, preferably alkyl, more preferably methyl.

Compounds exemplified by Formula (I) include, but are not limited to:
3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide
3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide
3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Compounds of Formula (I) for use in the methods of the present invention include those noted above and:

tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

The term "excessive IL-1b convertase activity" is used herein to mean an excessive expression of the protein, or activation of the enzyme.

The term "C_{1-6} alkyl" or "alkyl" is used herein to mean both straight and branched chain radicals of 1 to 6 carbon atoms, unless the chain length is otherwise specified, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and the like.

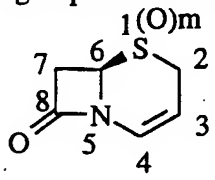
The term "heteroaryl" (on its own or in any combination, such as "heteroaryloxy", or "heteroaryl alkyl") is used herein to mean a 5-10 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, pyrazole, furan, thiophene, quinoline, isoquinoline, quinazolinyl, pyridine, pyrimidine, oxazole, oxadiazole, tetrazole, thiazole, thiadiazole, triazole, imidazole, or benzimidazole.

The term "aryl" (on its own or in any combination, such as "aryloxy", or "arylalkyl") is used herein to mean a phenyl and naphthyl ring.

The term "cycloalkyl" is used herein to mean cyclic radicals, preferably of 3 to 7 carbons, including but not limited to cyclopropyl, cyclopentyl, cyclohexyl, and the like.

The term"halo" or "halogens", is used herein to include, unless otherwise specified, chloro, fluoro, bromo and iodo.

For purposes herein the "core" group for Formula (I) is numbered as follows:



The present invention is to the inhibition of ICE and ICE-like proteases by compounds of Formula (I). What is meant by the term "ICE-like proteases" are fragment, homologs, analogs and derivatives of the polypeptides Interleukin-1 b converting enzyme (or convertase). These analogs are structurally related to the ICE family. They generally encode a protein (s) which exhibits high homology to the human ICE over the entire sequence. Preferably, the pentapeptide QACRG is conserved. The ICE like proteases , which may include many natural allelic variants (such as substitutions, deletion or addition of nucleotides) does not substantially alter the function of the encoded polypeptide. That is they retain essentially the same biological function or activity as the ICE protease, although it is recognized that the biological function may be enhanced or reduced activity. The suitable activity is not IL-1b convertase activity, but the ability to induce apoptosis or involved in programmed cell death in some manner. Suitable ICE like proteases encompasses within this invention are those described in PCT US94/07127 filed 23 June 1994, Attorney Docket No.: 325800-184; and in USSN 08/334,251, filed 1 November 1994, Attorney Docket No.: 325800-249 whose disclosures are incorporated herein by reference in their entirety.

The term "blocking or inhibiting, or decreasing the production of IL-1b and/or TNF" as used herein refers to:

a) a decrease of excessive levels, or a down regulation, of the cytokine in a human to normal or sub-normal levels by inhibition of the *in vivo* release of the cytokine; or

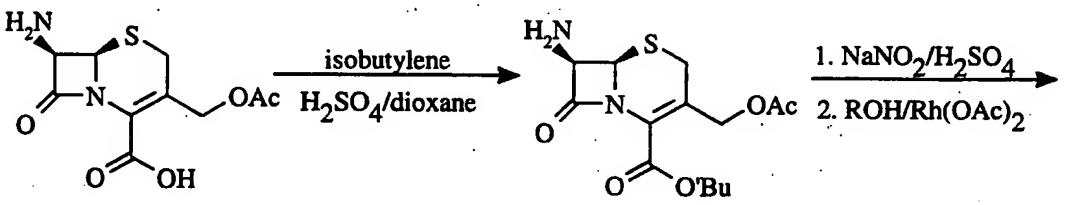
b) a down regulation, at the genomic level, of excessive *in vivo* levels of the cytokine (IL-1 or TNF) in a human to normal or sub-normal levels; or

c) a down regulation, by inhibition of the direct synthesis of the cytokine (IL-1, or TNF) as a postranslational event; or

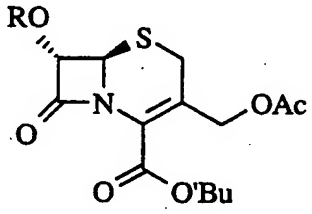
d) a down regulation, at the translational level, of excessive *in vivo* levels of the cytokine (IL-1, or TNF) in a human to normal or sub-normal levels.

The blocking or inhibiting, or decreasing the production of IL-1b and/or TNF is a discovery that the compounds of Formula (I) are inhibitors of the cytokines, IL-1 and TNF is based upon the effects of the compounds of Formulas (I) on the production of the IL-1 and TNF in *in vitro* and *in vivo* assays which are well known and recognized in the art, some of which are described herein.

Compound of the present invention may be synthesized by methods well known in the art, such as those described by the procedures of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513 whose disclosure is incorporated herein by reference. Alternatively, compounds of Formula (I) may be made in accordance with the schemes illustrated below.



I II



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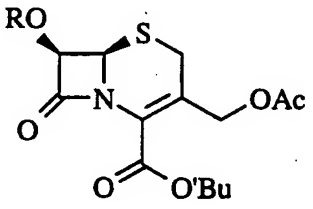

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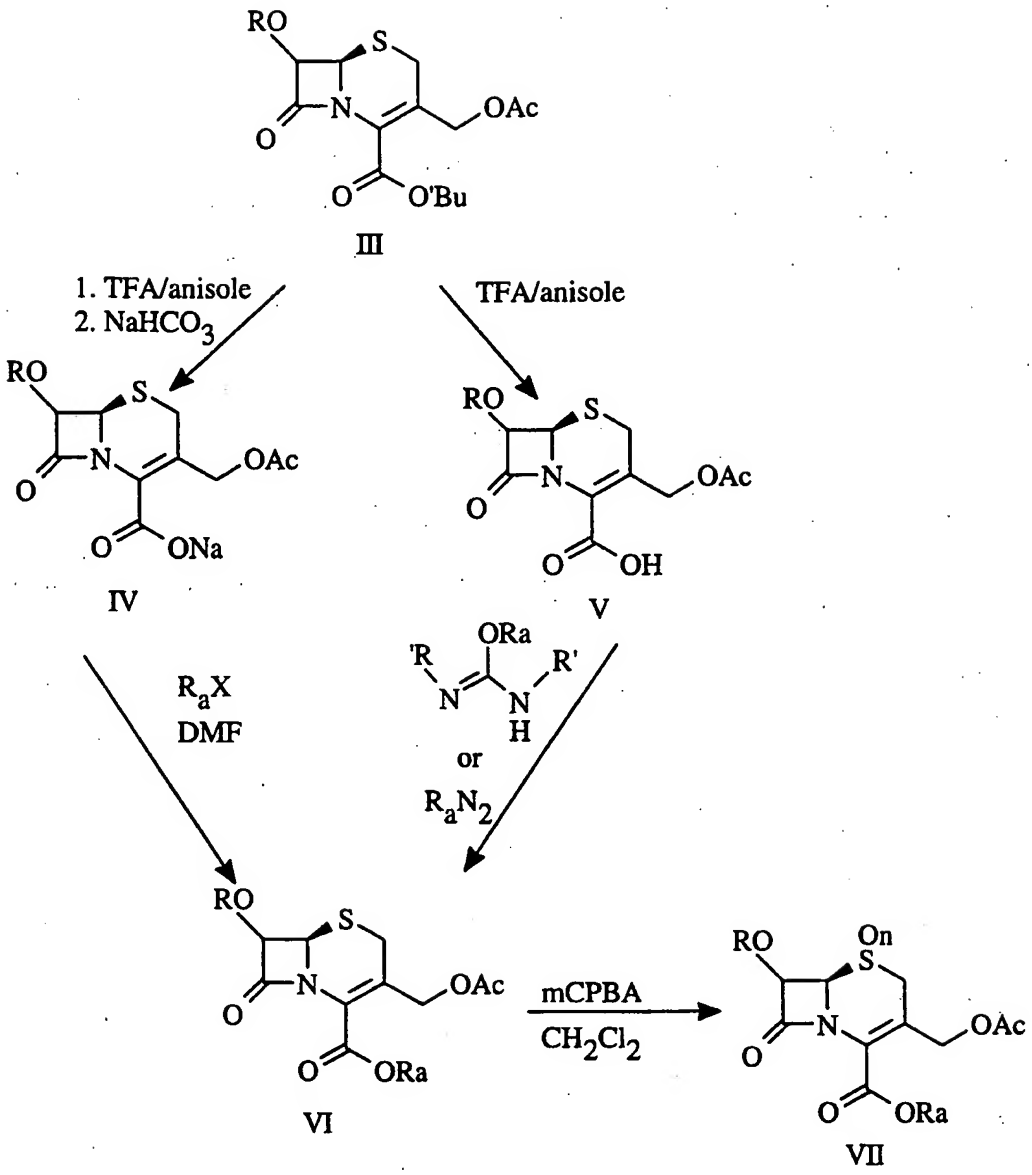
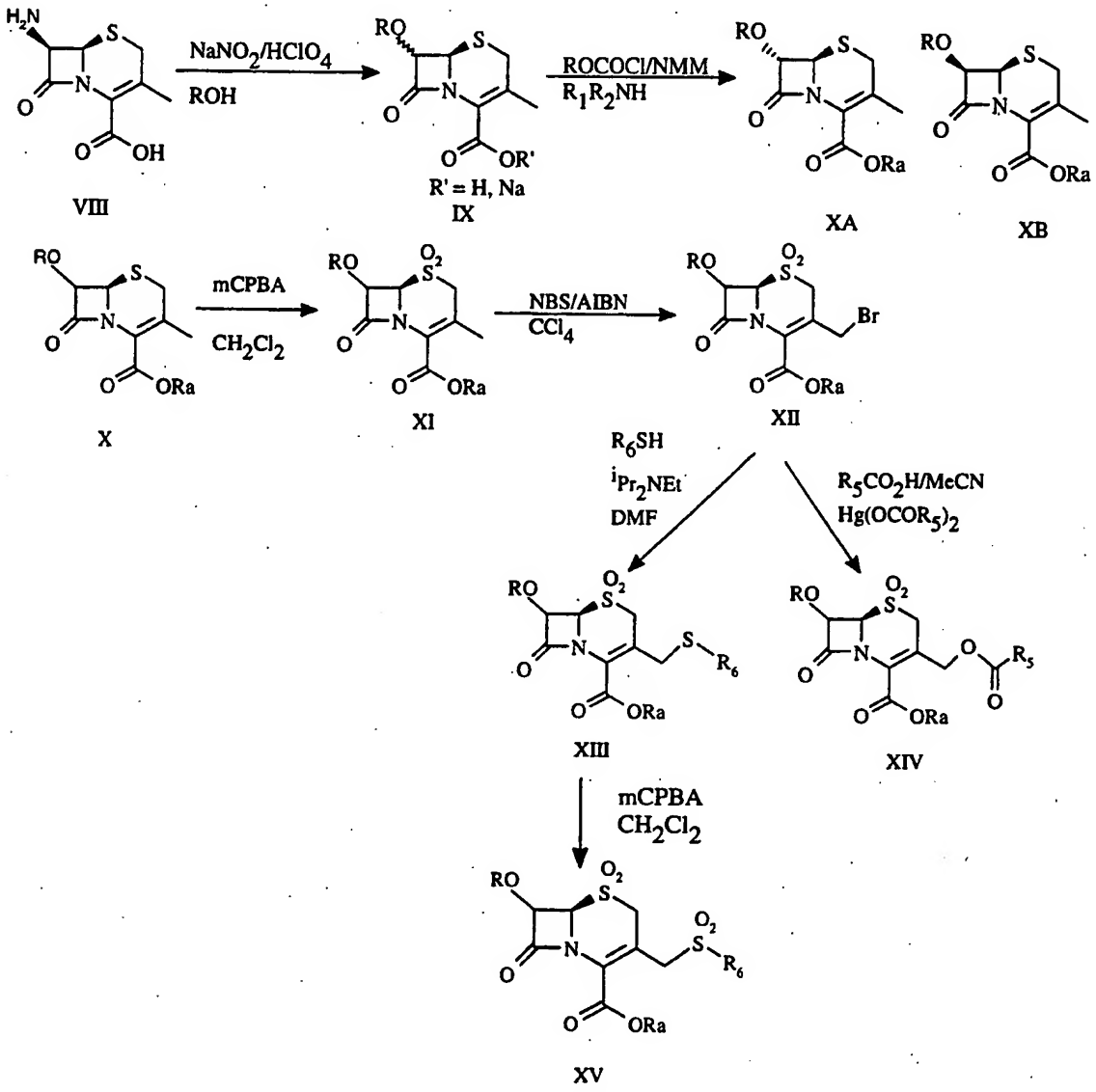


Scheme 1

The t-Butyl ester, 2-Scheme I, is synthesized by treating commercially available 7-aminocephalosporonic acid (1-Scheme I) with isobutylene and sulfuric acid in dioxane. Following the procedure of Doherty et al. (*J. Med. Chem.* 1990, **33**, 2513-2521, which is incorporated herein by reference), 7-alkoxy substituted 3a-Scheme I and 3b-Scheme I are produced as a separable mixture. Deprotection of 3-Scheme I with trifluoroacetic acid/anisole at 0°C gives the free acid 5-Scheme I or the sodium salt 4-Scheme I upon titration with aqueous sodium bicarbonate. Benzyl halide alkylations of IV in DMF give esters 6-Scheme I. Treatment of 5-Scheme I with diazo derivatives (Braun et al. *J. Am. Chem. Soc.* 1958, **80**, 359-363,which is incorporated herein by reference) or with alkoxyisoureas (Schmidt et al. *Justus Liebigs Ann. Chem.* 1965, **685**,

161-166,which is incorporated herein by reference) yields various alkylester derivatives (6-Scheme I). Finally, sulfone or sulfoxides 7-Scheme I are obtained by m-chloroperoxybenzoic acid or oxone oxidation of 6-Scheme I.



VIII IX XA XB X XI XII XIII XIV XV

Scheme 2

Alkoxy derivative 9-Scheme 2 is obtained in one step from 8-Scheme 2 by treatment with $NaNO_2$ and the alcohol in perchloric acid (Alpegiani et al. US 5,254,680, which is incorporated herein by reference). Ester 10-Scheme I is formed by esterification of 9-Scheme 2 by procedures described for 6-Scheme 1; m-chloroperoxybenzoic acid or oxone oxidation of 10-Scheme I yields 11-Scheme 2. The following derivatives can be synthesized according to procedures outlined by Alpegiani et al. *J. Med. Chem.* 1994, **37**, 4003-4019, which is incorporated herein by reference:

exposure of 11-Scheme 2 to N-bromosuccinimide under radical conditions gives the 3-bromomethyl derivative 12-Scheme 2; 13-Scheme 2 and 14-Scheme 2 are accessible through displacement of the bromide by aromatic thiols and mercuric acetate derivatives. Sulfones 15-Scheme 2 are obtained by oxidation of their corresponding thioethers (13-Scheme 2).

SYNTHETIC CHEMISTRY

Without further elaboration, it is believed that one skilled in the art can, using the preceding descriptions, utilize the present invention to its fullest extent. The following examples further illustrate the synthesis of compounds of this invention. The following examples are, therefore, to be construed as merely illustrative and not a limitation of the scope of the present invention in any way.

Temperatures are recorded in degrees centigrade unless otherwise noted.

Example 1

tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

The title compound was prepared according to the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513.

Example 2

tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513, the title compound is isolated as a minor component of the final mixture.

Example 3

3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) 3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate

To tert-Butyl (6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate (prepared by the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513) (1.0 g, 2.9 mmol) and anisole (3.2 mL, 29 mmol) was added trifluoroacetic acid (16 mL) at 0_C under Ar. The solution was stirred for 30 min, and concentrated *in vacuo*.

The residue was dissolved in methylene chloride (50 mL), washed with water, washed with brine, dried ($MgSO_4$), filtered and concentrated *in vacuo* to an oil. The residue was dissolved in ethyl acetate (30 mL), and water (30 mL) was added. A solution of saturated sodium bicarbonate was dropped in until the aqueous layer reached pH 7. The aqueous layer was separated, and the procedure was repeated with

another 30 mL of water. The aqueous layers were combined and freeze-dried to afford a yellow solid (870 mg).

To the sodium salt (187 mg) in dimethylformamide (6 mL) was added 3,4-dichlorobenzyl chloride (168 uL) under Ar and the solution was stirred for 22 h. To the solution was added ether, the mixture was washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. The oil was purified by flash chromatography (silica gel, 25-45% ethyl acetate/hexanes) to yield a 3:2 mixture of the title compound and the Æ2 regioisomer (85 mg, 30% overall yield). ^1H NMR(400 MHz, $CDCl_3$) d 7.1-7.6 (m, 3H), 6.46, 4.5-5.3 (m, 6H), 3.3-3.7 (m, 5H), 3.55 (m, 3H).

b) 3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

To the ester (83 mg, 186 umol) of Example 2(a) in methylene chloride (3 mL) was added 85% m-chloroperoxybenzoic acid (114 mg, 558 umol) and the solution was stirred for 4 h. To the solution was added 20% sodium metabisulfite, followed by saturated sodium bicarbonate and the mixture was extracted with methylene chloride. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 40-50% ethyl acetate/hexanes) to yield the title compound (75 mg, 84%). MS(ES^+) m/e 478 $[M+H]^+$.

Example 4

tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide

a) tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate

Following the procedure of Doherty *et al.*, *J. Med. Chem.*, 1990, 33, 2513, except substituting ethylene glycol for methanol, the title compound was prepared. ^1H NMR(400 MHz, $CDCl_3$) d 4.93 (d, J=13.7 Hz, 1H), 4.73 (d, J=13.7 Hz, 1H), 4.70 (s, 1H), 4.61 (s, 1H), 3.81 (br s, 4H), 3.58 (d, J=18.4 Hz, 1H), 2.07 (s, 3H), 1.54 (s, 9H).

b) tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethyloxy)-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3(b), except substituting the title compound of Example 4(a) for the ester of Example 3(a), the title compound was prepared. MS(ES^-) m/e 404 $[M-H]^-$.

Example 5

Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) (6R,7S)-3-Acetoxymethyl-7-methoxy-3-cephem-4-carboxylic acid

The intermediate sodium salt (85 mg) from Example 3(a) was purified by flash chromatography (0.5% acetic acid/10% methanol/methylene chloride) to yield the free acid (50 mg). ^{1}H NMR(400 MHz, 2:1 $CDCl_3/CD_3OD$) d 4.87 (d, J=12.6 Hz, 1H), 4.73 (d, J=12.6 Hz, 1H), 4.61 (s, 1H), 4.40 (d, J=1.7 Hz, 1H), 3.49 (d, J=17.8 Hz, 1H), 3.44 (s, 3H), 3.17 (d, J=17.8 Hz, 1H), 2.09 (s, 3H).

b) Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate

To the acid of Example 5(a) (48 mg, 167 umol) in tetrahydrofuran (3 mL) was added a 0.1 M ethereal solution of diazomethane (10 mL) at 0°C. The solution was stirred for 15 min, and quenched with an excess of acetic acid. The mixture was diluted with methylene chloride, washed with saturated sodium bicarbonate, concentrated in vacuo and purified by flash chromatography (silica gel, 15-25% ethyl acetate/hexanes) to yield the title compound (25 mg, 52%). ^{1}H NMR(400 MHz, $CDCl_3$) d 4.97 (d, J=13.2 Hz, 1H), 4.76 (d, J=13.2 Hz, 1H), 4.69 (s, 1H), 4.51 (s, 1H), 3.89 (s, 3H), 3.58 (d, J=18.3 Hz, 1H), 3.55 (s, 3H), 3.32 (d, J=18.3 Hz, 1H), 2.07 (s, 3H).

c) Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3(b), except substituting the title compound of Example 5(b) for the ester of Example 3(a), the title compound was prepared. MS(ES^-) m/e 332 $[M-H]^-$.

Example 6

Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting benzyl bromide for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^+) m/e 410 $[M+H]^+$.

Example 7

3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 70% 3,4-dimethylbenzyl chloride (30% 2,3-dimethylbenzyl chloride) for 3,4-dichlorobenzyl chloride, the title compound was prepared as a 1:1 mixture with 2,3-dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide. MS(ES$^+$) m/e 438 $[M+H]^+$.

Example 8

4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 4-nitrobenzyl bromide for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES$^-$) m/e 453 $[M-H]^-$.

Example 9

3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide

Following the procedure of Example 3, except one equivalent of m-chloroperoxybenzoic acid is used. MS(ES$^-$) m/e 460 $[M-H]^-$.

Example 10

3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide for tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide, the title compound was prepared. MS(ES$^-$) m/e 478 $[M-H]^-$.

Example 11

4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 4-iodobenzyl chloride for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES$^+$) m/e 536 $[M+H]^+$.

Example 12

3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 3-iodobenzyl chloride for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^-) m/e 534 $[M-H]^-$.

Example 13

3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting 3-iodo-4-methylbenzyl chloride for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^+) m/e 550 $[M+H]^+$.

Example 14

3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3, except substituting the title compound of Example 4(a) for tert-butyl (6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate. MS(ES^-) m/e 506 $[M-H]^-$.

Example 15

3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxyl-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 16, except substituting n-butanol for ethylene glycol, the title compound was prepared. MS(ES^-) m/e 518 $[M-H]^-$.

Example 16

3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 16, except substituting ethanol for 3,4-dichlorobenzyl chloride, the title compound was prepared. MS(ES^-) m/e 490 $[M-H]^-$.

Example 17

3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) 3,4-Dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate

To a solution of (6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylic acid (1 g) in ethyl acetate (30 mL) is added water (30 mL). A solution of saturated sodium bicarbonate is dropped in until a pH of 7 in the aqueous layer is obtained. The aqueous layer is separated and lyophylized to afford sodium-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate (670 mg).

To sodium-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate (312 mg) in dimethylformamide (2 mL) is added 3,4-dichlorobenzyl chloride (500 uL), and the solution was stirred for 24 h. To the solution was added water and the solution was extracted with ether. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 15-25% ethyl acetate/hexanes) to yield 3,4-dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate (148 mg). ^1H NMR(250 MHz, $CDCl_3$) d 7.2-7.6 (m, 3H), 5.23 (s, 2H), 4.67 (s, 1H), 4.50 (s, 1H), 3.4-3.6 (m, 4H), 3.19 (d, J=18.4 Hz, 1H), 2.10 (s, 3H).

b) 3,4-Dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3b, except substituting 3,4-dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate for the ester of example 2a, the sulfone was prepared. ^1H NMR(250 MHz, $CDCl_3$) d 7.2-7.6 (m, 3H), 5.21 (s, 2H), 5.13 (s, 1H), 4.62 (s, 1H), 3.88 (d, J=18.4 Hz, 1H), 3.66 (d, J=18.4 Hz, 1H), 3.56 (s, 3H), 2.10 (s, 3H).

c) 3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide.

To the sulfone of Example 17b (91 mg) in carbon tetrchloride (4 mL) is added AIBN (5 mg) and N-bromosuccinimide (44 mg), and solution was reluxed under argon for 3 h. the reaction mixture was cooled, saturated sodium bicarbonate was added, and the mixture was extracted with methylene chloride. The organic extract was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 15-35% ethyl acetate/hexanes) to yield the title compound (45 mg). MS(ES^-) m/e 496 $[M-H]^-$.

Example 18

3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

a) 3,4-Dichlorobenzyl-(6R,7S)-3-phenylthiomethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

To the bromide of Example 17 (110 mg) in dimethylformamide (15 mL) at 0°C was added thiophenol (25 uL) and N,N-diisopropyl-N-ethylamine (42 uL). The solution was stirred until the disappearance of starting material. Water was added, and the solution was extracted with ether. The organic extract concentrated *in vacuo*, and the residue was purified by flash chromatography (silica gel, ethyl acetate/hexanes) to yield the title compound. MS(ES$^+$) m/e 528 $[M+H]^+$.

b) 3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 3b, except substituting 3,4-dichlorobenzyl-(6R,7S)-7-methoxy-3-methyl-3-cephem-4-carboxylate for the ester of example 2a, the sulfone was prepared. MS(ES$^+$) m/e 560 $[M+H]^+$.

Example 19

3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 18a, except substituting 5-methyl-(1,3,4-oxadiazol)-2-mercaptan for thiophenol, the title compound was prepared. MS(ES$^+$) m/e 534 $[M+H]^+$.

Example 20

3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 18a, except substituting (1-methyltetrazole)-5-mercaptan for thiophenol, the title compound was prepared. MS(ES$^+$) m/e 534 $[M+H]^+$.

Example 21

3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

Following the procedure of Example 18a, except substituting (1,2,3-triazole)-4-mercaptan for thiophenol, the title compound was prepared. MS(ES$^+$) m/e 519 $[M+H]^+$.

BIOLOGICAL ASSESSMENTS:

Assay I - DNA Ladder:

The present invention utilizes a model that measures apoptosis, by measuring the production of DNA ladders visualized on agarose gels. The observation of DNA ladders has been a hallmark of the apoptosis response for many years. The model used in our studies is the production of apoptosis in human monocytic HL-60 cells by the anti-cancer ether lipid 1-O-octadecyl-2-O-methyl-*sn*-3-phosphocholine (ET-18-OCH_3) and tumor necrosis factor a (TNF). The production of DNA ladders by ET-18-OCH_3 was recently reported (Mollinedo et al. Biochem. Biophys. Res. Commun., 192: 603-609 (1993)) and confirmed in house. The general method is to treat HL-60 cells with 6 μM ET-18-OCH_3 or 10 units of TNF for 24 hours, followed by extraction of small molecular weight DNA and removal of protein and RNA. The DNA is separated on a agarose gel and visualized with ethidium bromide staining. An internal standard is added to the cells just prior to extraction and preparation of DNA. Drugs are provided to cells 10 minutes prior to the apoptotic insult. This method provides a qualitative assessment of the ability of compounds to inhibit apoptosis.

Cell Conditions

- HL-60 cells (American Type Cell Culture) were grown and kept at log phase in RPMI 1640 w/L-glutamine and 10 % heat inactivated Fetal Bovine Serum (RPMI complete).
- On the day of the experiment, the desired number of cells (for example, 5 x 10^6 cells/treatment group) were resuspend in RPMI complete to give a final cell concentration of approximately 0.5 x 10^6 cells/ml. For each treatment group, 10 mls of cell suspension were placed in a culture flask. Cells were incubated for 2 hours at 37°C.

Exposures:

- For typical expose to ET-18-OCH_3, a 100 mM ET-18-OCH_3 stock solution in $CHCl_3$ was prepared, then diluted in RPMI complete to 600 μM. Then 100 μl of 600 μM ET-18-OCH_3 was added into 10 ml treatment group yielding a final concentration of 6 μM. The cell suspensions are then incubated overnight (18 hours). For a typical exposure to TNF, 300 to 3000 units of TNF were added to 10 ml of cell suspension.
- Cells were pretreated with desired agents (ICE compounds, etc.) 10 minutes prior to ET-18-OCH_3 or TNF addition. ICE compounds stocks were in DMSO. 50 μl of compound or DMSO vehicle was added to the 10 ml treatment groups yielding the final concentration of compound and 0.5 % DMSO.

DNA Extraction:

- Cells were spun (400 x g, 5 min) and washed 2x in 10 mLs PBS.
- Cells were lysed by resuspending them into 200 µL of cold, sterile detergent buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.2% Triton X-100) and transferring the approximately 250 µL volume to sterile, 1.5 mL eppendorf tubes on ice. Then, tubes were incubated for 30 min. at 4°C, with mild shaking.
- Tubes were spun in a Microfuge for 15 min., the supernatant collected, taking care to avoid the cellular debris.
- The supernatants were incubated with 75 µg/mL RNAseA for 1 hr at 37°C then incubated with 200 µg/mL ProteinaseK and 0.5 % SDS [final] for 1 hr at 37°C.
- Ten µl of a 300 bp DNA was added as an internal standard to observe extraction efficiency.
- Supernatants were extracted twice with equal volume (200-300 µL) of cold, buffer saturated phenol (add phenol, vortex 15 seconds, microfuge 2 min., collect the top aqueous layer, avoiding the organic waste in between the two phases), once with 200 µL Phenol/Chloroform/Isoamyl alcohol 25:24:1 (v/v) and once with 100 µL Chloroform (100µL/sample is retrieved).
- Add 10 µL of sterile 3M NaCl (300 mM [final]) to the 100 µL sample and 200 µL of cold ethanol, vortex well and let stand overnight at -20°C.
- Samples were spun (Microfuge) 15 min and all but 25 µl of the ethanol was carefully removed. The DNA pellets were dried and resuspended in 30 µL of sterile 10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA and 10 µL of gel loading Buffer. Load 20 µl/well.
- A DNA standard (for example, Sigma # D 5042, 123 bp ladder) was run on each gel.
- Samples were run on 1-2 % agarose gel with TBE buffer (5X TBE = 54 g Tris Base, 27.5 g Boric acid, 20ml 0.5 M EDTA, pH 8.0) with ethidium bromide added, for example for 90 - 120 min at 100 V, 50 mA.
- The resulting gels were visualized under UV light and the results recorded in a captured image.

Results

In HL-60 cells, treatment with ET-18-OCH_3 or TNF induced an apoptotic response that was prominent after 24 hours. Pretreatment with 50 µM of the compound of Example 3 was found to completely block the apoptotic response to both ET-18-OCH_3 or TNF (Table 1). Pretreatment with 5 µM of the compound of Example 3 was not effective over the 24 hour experiment. Addition of IL-1b (10 nM) had no effect on the ability of the compound of Example 3 to block apoptosis, suggesting that its primary mechanism of action is not inhibition of IL-1 production. These data support

that the compound of Example 3 blocks apoptosis by a novel mechanism of action, i.e., by inhibiting the activity of ICE and ICE-like proteases.

Table 1. Effect of Compound on Apoptosis

Drug	Concentration	Apoptotic Signal	Concentration	Presence of Apoptosis
None		ET-18-OCH_3	6 μM	yes
Example 3	50 μM	ET-18-OCH_3	6 μM	no
Example 3	5 μM	ET-18-OCH_3	6 μM	yes
None		TNF	270 U/ml	yes
Example 3	50 μM	TNF	270 U/ml	no

Assay II: Inhibition of ICE

Source of Enzyme

Human ICE was cloned and expressed in *E. coli* as its inactive precursor (p45) bearing a hexa-His flag on its *amino*-terminal end. Following harvesting, the cells were lysed, centrifuged, and the pellet containing the p45 solubilized with phosphate buffered 7 M urea at pH 7.5. The flagged p45 was applied to a Ni-nitrilo-acetic acid column, washed, and eluted with 300 mM imidazole. This yielded a highly enriched proenzyme preparation (390% pure p45). Catalytic autoproteolytic activation to p10/p20 dimer was achieved by concentrating the p45 on a Centricon ultrafiltration membrane (Amicon) at 10 _C for several hours. The formation of the catalytic subunits (p10 and p20) in activated samples was demonstrated by correlating time-dependent generation of ICE activity with p10/p20 signals in Western blots and by reversed-phase HPLC. Formation of authentic p10 and p20 was also confirmed by *N*-terminal sequence and MALD-mass spectral analyses of samples purified by reversed-phase HPLC. The activated enzyme was stored frozen at –80 _C.

Assay Protocol

ICE was assayed at 25 _C using the fluorogenic tetrapeptide substrate *N*-acetyl-L-tyrosyl-L-valyl-L-alanyl-L-aspartyl-7-amido-4-methylcoumarin (Ac-**YVAD**-AMC). The assays were conducted at pH 7.5 in a buffered system containing 25 mM Hepes, 10% sucrose, 0.1% CHAPS, and 2 mM DTT. The concentration of substrate was fixed at 25 uM. Fluorescence of the liberated 7-amino-4-methylcoumarin was continuously monitored at 460 nm following excitation at 335 nm.

Compound Testing

Compounds of Formula (I) were tested at a single dose of 100 uM following a 30 to 60-min preincubation with enzyme. The assay was initiated by the addition of 25 uM substrate (Ac-**YVAD**-AMC) and activity was monitored as described above.

Representative compounds of Formula (I), as exemplified by Examples 1 to 7 and 9 demonstrated positive inhibitiory activity in this assay ranging from about 36% to about 96%.

Assay III: Inhibition of ICE

ICE was assayed at 25 _C in 96-well plates using the fluorogenic tetrapeptide substrate *N*-acetyl-L-tyrosyl-L-valyl-L-alanyl-L-aspartyl-7-amido-4-methylcoumarin (Ac-**YVAD**-AMC). The assays were conducted at pH 7.5 in a buffered system containing 25 mM Hepes, 10% sucrose, 0.1% CHAPS, and 20-50 uM DTT. The concentration of substrate was fixed at 20 uM. Fluorescence of the liberated 7-amino-4-methylcoumarin was continuously monitored at 460 nm following excitation at 360 nm.

Compound Testing

Compounds were tested at a single dose of 50 to 100 uM. Activity was monitored as described above over a 30 to 60-minute time period following the simultaneous addition of substrate and inhibitor to initiate the reaction. The progress curves thus generated were fit by computer to Eq. 1 in order to assess potency and time-dependency:

$$v = \frac{(V_o(1 - e^{-k_{obs}t})}{k_{obs}} \qquad (1)$$

Representative compounds of formula (I) have demonstrated positive inhibitory activity in the above noted assay:

3,4-Dichlorobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

tert-Butyl 7-alpha-methoxycephalosporanate sulfone

3,4-Dichlorobenzyl-(6R,7S)-3-(1-methyltetrazol-5-yl)thiomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-(phenylsulfonyl)methyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[2-methyl(1,3,4-oxadiazol-5-yl)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl (6R,7S)-3-(1,2,3-triazol-5-yl)thiomethyl-7-methoxy-3-cephem-4-carboxylate 1,1-dioxide

3,4-Dichlorobenzyl-5,5-dioxo-7-alpha-[2-hydroxyethyloxy]-cephalosporanate
3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
Benzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
[(3,4)- and (2,3)-]Dimethylbenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide N-3,4-Dichlorobenzyl-N-methyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxamide-1,1-dioxide
(6R,7S)-4-Iodobenzyl--7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodo-4-methylbenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

METHODS OF TREATMENT

For therapeutic use the compounds of the present invention will generally be administered in a standard pharmaceutical composition obtained by admixture with a pharmaceutical carrier or diluent selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally in the form of tablets containing such excipients as starch or lactose, or in capsule, ovules or lozenges either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. They may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The choice of form for administration as well as effective dosages will vary depending, inter alia, on the condition being treated. The choice of mode of administration and dosage is within the skill of the art.

The compounds of the present invention, particularly those noted herein or their pharmaceutically acceptable salts which are active when given orally, can be formulated as liquids, for example syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in a suitable liquid carrier(s) for example, ethanol, glycerin, non-aqueous solvent, for example polyethylene glycol, oils, or water with a suspending agent, preservative, flavouring or colouring agent.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses, silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule. Preferably the composition is in unit dose form such as a tablet or capsule.

Typical parenteral compositions consist of a solution or suspension of the compound or pharmaceutically acceptable salt in a sterile aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil. Alternatively, the solution can be lyophilized and then reconstituted with a suitable solvent just prior to administration.

A typical suppository formulation comprises a compound or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent such as polymeric glycols, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats.

The pharmaceutically acceptable compounds of the invention will normally be administered to a subject in a daily dosage regimen. For a patient this may be, for example, from about .001 to about 100mg/kg, preferably from about 0.001 to about 10mg/kg animal body weight. A daily dose, for a larger mammal is preferably from about 1 mg to about 1000 mg, preferably between 1 mg and 500 mg or a pharmaceutically acceptable salt thereof, calculated as the free base, the compound being administered 1 to 4 times per day. Unit dosage forms may contain from aobut 25µg to about 500mg of the compound.

There are many diseases and conditions in which dysregulation of apoptosis plays an important role. All of these conditions involve undesired, deleterious loss of specific cells with resulting pathological consequences.

Bone remodeling involves the initial resorption by osteoclasts, followed by bone formation by osteoblasts. Recently, there have been a number of reports of apoptotic events occurring during this process. Apoptotic events have been observed in both the bone forming and bone resorbing cells *in vitro* and indeed at the sites of these remodeling units *in vivo.*

Apoptosis has been suggested as one of the possible mechanisms of osteoclast disappearance from reversal sites between resorption and formation. TGF-ß1 induces apoptosis (approx. 30%) in osteoclasts of murine bone marrow cultures grown for 6 days *in vitro* . (Hughes, et al., *J. Bone Min. Res.* **9** , S138 (1994)). The anti-resorptive

bisphosphonates (clodronate, pamidronate or residronate) promote apoptosis in mouse osteoclasts *in vitro* and *in vivo* . (Hughes, et al., supra at S347). M-CSF, which has previously been found to be essential for osteoclast formation can suppress apoptosis, suggesting not only that maintenance of osteoclast populations, but also that formation of these multinucleated cells may be determined by apoptosis events. (Fuller, et al., *J. Bone Min. Res.* **8** , S384 (1993); Perkins, et al., *J. Bone Min. Res.* **8** , S390 (1993)). Local injections of IL-1 over the calvaria of mice once daily for 3 days induces intense and aggressive remodeling. (Wright, et al., *J. Bone Min. Res.* **9** , S174 (1994)). In these studies, 1% of osteoclasts were apoptotic 1 day after treatment, which increased 3 days later to 10%. A high percentage (95%) of these apoptotic osteoclasts were at the reversal site. This data suggests that ICE or ICE-like homologues are functionally very important in osteoclast apoptosis.

Therefore, one aspect of the present invention is the promotion of apoptosis in osteoclasts as a novel therapy for inhibiting resorption in diseases of excessive bone loss, such as osteoporosis, using compounds of Formula (I) as defined herein.

Apoptosis can been induced by low serum in highly differentiated rat osteoblast-like (Ros 17/2.8) cells (Ihbe, et al., (1994) *J. Bone Min. Res.* **9**, S167)). This was associated with a temporal loss of osteoblast phenotype, suggesting that maintenance of lineage specific gene expression and apoptosis are physiologically linked. Fetal rat calvaria derived osteoblasts grown *in vitro* undergo apoptosis and this is localized to areas of nodule formation as indicated by *in situ* end-labeling of fragmented DNA. (Lynch, et al., (1994) *J. Bone Min. Res.* **9**, S352). It has been shown that the immediate early genes c-fos and c-jun are expressed prior to apoptosis; c-fos and c-jun-Lac Z transgenic mice show constitutive expression of these transcription factors in very few tissues, one of which is bone (Smeyne, et al., (1992) *Neuron.* **8**, 13-23; and Morgan, J. (1993) Apoptotic Cell Death: Functions and Mechanisms. Cold Spring Harbor 13-15th October). Apoptosis was observed in these animals in the epiphyseal growth plate and chondrogenic zones as the petula ligament calcifies. Chondrogenic apoptosis has also been observed in PTHRP-less mice and these transgenics exhibit abnormal endochondral bone formation (Lee, et al., (1994) *J. Bone Min. Res.* **9**, S159). A very recent paper examined a human osteosarcoma cell line which undergoes spontaneous apoptosis. Using this cell line, LAP-4, but not ICE, could be detected and *in vitro* apoptosis could be blocked by inhibition or depletion of LAP-4 (Nicholson, et al., (1995) *Nature* **376**, 37-43). Thus, apoptosis may play a role in loss of osteoblasts and chondrocytes and inhibition of apoptosis could provide a mechanism to enhance bone formation.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to enhance bone formation using compounds of Formula (I) as defined herein.

Osteoarthritits (OA) is a degenerative disease characterized by progressive erosion of articular cartilage. Chondrocytes are the single cell-type found in articular cartilage and perturbations in metabolism of these cells may be involved in the pathogenesis of OA. Injury to cartilage initiates a specific reparative response which involves an increase in the production of proteoglycan and collagen in an attempt to reestablish normal matrix homeostasis. However, with the progress of the disease, the 3-dimensional collagen network is disrupted and cell death of chondrocytes occurs in OA lesions (Malemud, et al.: Regulation of chondrocytes in osteoarthritis. In: Adolphe, M. ed. Biological Regulation of Chondrocytes. Boca Raton:CRC Press, 1992, 295-319). It has been shown that in OA, chondrocytes adjacent to cartilage defects express high levels of bcl-2 (Erlacher, et al., (1995) *J. of Rheumatology*, 926-931). This represents an attempt to protect chondrocytes from apoptosis induced by the disease process.

Protection of chondrocytes during early degenerative changes in cartilage by inhibition of apoptosis may provide a novel therapeutic approach to this common disease. Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat osteoarthritis, using compounds of Formula (I) as defined herein.

Recent evidence shows that chronic, degenerative conditions of the liver are linked to hepatocellular apoptosis. These conditions include chemical-, infectious- and immune/inflammatory-induced hepatocellular degeneration. Apoptosis of liver cells has been observed in liver degenerative states induced by a variety of chemical agents, including acetaminophen (Ray, et a l.,(1993) *FASEB. J.* **7**, 453-463), cocaine (Cascales, et al., (1994) *Hepatology* **20**, 992-1001) and ethanol (Baroni, etal., (1994) *J. Hepatol.* **20**, 508-513). Infectious agents and their chemical components that have been shown to induce apoptosis include hepatitis ((Hiramatsu, et al., (1994) *Hepatology* **19**, 1354-1359; Mita, et al., (1994) *Biochem. Biophys. Res. Commun.* **204**, 468-474)), tumor necrosis factor and endotoxin . (Leist, et al., (1995) *J. Immunol.* **154**, 1307-1316; and Decker, K. (1993) *Gastroenterology* **28(S4)**, 20-25). Stimulation of immune / inflammatory responses by mechanisms such as allograft transplantation and hypoxia followed by reperfusion have been shown to induce apoptosis of hepatocytes (Krams, et al., (1995) *Transplant. Proc.* **27**, 466-467). Together, this evidence supports that hepatocellular apoptosis is central to degenerative liver diseases.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat degenerative liver diseases., using compounds of Formula (I) as defined herein.

Apoptosis is recognized as a fundamental process within the immune system where cell death shapes the immune system and effects immune functions. Apoptosis also is implicated in viral diseases (e.g AIDS). Recent reports indicate that HIV infection may produce an excess of apoptosis, contributing to the loss of $CD4^+$ T cells. Of additional interest is the observation that APO-1/Fas shares sequence homology with HIV-1 gp120.

Therefore, another aspect of the present invention is the inhibition of apoptosis as a novel therapy to treat viral diseases, using compounds of Formula (I) as defined herein.

Additional therapeutic directions and other indications in which inhibition of apoptotic cysteine proteases is of therapeutic utility, along with relevant citations in support of the involvement for apoptosis in each indication, are presented below in Table 1.

Table 1: Therapeutic Indications Related to Apoptosis

Indication	Citations
Ischemia / reperfusion	Barr et al., (1994) *BioTechnology* **12**, 487-493; Thompson, C. B. (1995) *Science* **267**, 1456-1462
Stroke	Barr et al supra; and Thompson, C., supra
Polycystic kidney disease	Barr et al., supra; and Mondain, et al., (1995) *ORL J. Otorhinolaryngol. Relat. Spec.* **57**, 28-32
Glomerulo-nephritis	Barr et al., supra
Osteoporosis	Lynch et al., (1994) *J. Bone Min. Res.* **9**, S352; Nicholson et al., (1995) *Nature* **376**, 37-43
Erythropoiesis / Aplastic anemia	Thompson, C., supra; Koury et al., (1990) *Science* **248**, 378-381

Chronic liver degeneration	Thompson, C., supra; Mountz et al., 1994) *Arthritis Rheum.* **37**, 1415-1420; Goldin et al., (1993) *Am. J. Pathol.* **171**, 73-76
T-cell death	Thompson, C., supra; Ameison et al., (1995) *Trends Cell Biol.* **5**, 27-32
Osteoarthritis - chondrocytes	Ishizaki et al., (1994) *J. Cell Biol.* **126**, 1069-1077; Blanco et al., (1995) *Am. J. Pathol.* **146**, 75-85
Male pattern baldness	Mondain et al., supra; Seiberg et al., (1995) *J. Invest. Dermatol.* **104**, 78-82; Tamada et al., (1994) *Br. J. Dermatol.* **131**, 521-524
Alzheimer's disease	Savill, J.,(1994) *Eur. J. Clin. Invest.* **24**, 715-723; Su et al., (1994) *Neuroreport* **5**, 2529-2533; Johnson, E., (1994) *Neurobiol. Aging* **15 Suppl. 2**, S187-S189
Parkinson's disease	Savill, J., supra; Thompson, C., supra
Type I diabetes	Barr et al., supra

The IL-1 and TNF inhibiting effects of compounds of the present invention are determined by the following *in vitro* assays:

Interleukin - 1 (IL-1)

Human peripheral blood monocytes are isolated and purified from either fresh blood preparations from volunteer donors, or from blood bank buffy coats, according to the procedure of Colotta *et al*, J Immunol, **132**, 936 (1984). These monocytes ($1x10^6$) are plated in 24-well plates at a concentration of 1-2 million/ml per well. The cells are allowed to adhere for 2 hours, after which time non-adherent cells are removed by gentle washing. Test compounds are then added to the cells for about 1hour before the addition of lipopolysaccharide (50 ng/ml), and the cultures are incubated at 37°C for an additional 24 hours. At the end of this period, culture super-natants are removed and clarified of cells and all debris. Culture supernatants are then immediately assayed for IL-1 biological activity, either by the method of Simon *et al.*, J. Immunol. Methods, **84**, 85, (1985) (based on ability

of IL-1 to stimulate a Interleukin 2 producing cell line (EL-4) to secrete IL-2, in concert with A23187 ionophore) or the method of Lee *et al.*, J. ImmunoTherapy, **6** (1), 1-12 (1990) (ELISA assay).

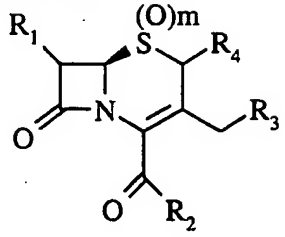
Tumour Necrosis Factor (TNF):

Human peripheral blood monocytes are isolated and purified from either blood bank buffy coats or platelet pheresis residues, according to the procedure of Colotta, R. *et al.*, J Immunol, **132**(2), 936 (1984). The monocytes are plated at a density of $1x10^6$ cells/ml medium/well in 24-well multi-dishes. The cells are allowed to adhere for 1 hour after which time the supernatant is aspirated and fresh medium (1ml, RPMI-1640, Whitaker Biomedical Products, Whitaker, CA) containing 1% fetal calf serum plus penicillin and streptomycin (10 units/ml) added. The cells are incubated for 45 minutes in the presence or absence of a test compound at 1nM-10mM dose ranges (compounds are solubilized in dimethyl sulfoxide/ethanol, such that the final solvent concentration in the culture medium is 0.5% dimethyl sulfoxide/0.5% ethanol). Bacterial lipopoly-saccharide (*E. coli* 055:B5 [LPS] from Sigma Chemicals Co.) is then added (100 ng/ml in 10 ml phosphate buffered saline) and cultures incubated for 16-18 hours at 37°C in a 5% CO_2 incubator. At the end of the incubation period, culture supernatants are removed from the cells, centrifuged at 3000 rpm to remove cell debris. The supernatant is then assayed for TNF activity using either a radio-immuno or an ELISA assay, as described in WO 92/10190 and by Becker *et al.*, J Immunol, 1991, **147**, 4307.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the are can, using the preceding description, utilize the present invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A compound of the formula:



(I)

wherein

R_1 is hydrogen, an optionally substituted alkoxy or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, $-OC(O)R_5$, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;

R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;

n is 0, or an integer having a value of 1 or 2;

or a pharmaceutically acceptable salt thereof; excluding the compounds tert-Butyl 7-alpha-methoxycephalosporanate sulfone; tert-Butyl 7-beta-methoxycephalosporanate sulfone; Methyl (6R,7S)-7-Methoxy-3-acetoxymethyl-3-cephem-4-carboxylic acid -1,1-dioxide; and Benzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide.

2. The compound according to Claim 1 wherein R_a is a benzyl moiety optionally substituted independently one or more times by hydroxy, halogen, alkyl, or alkoxy.

3. The compound according to Claim 1 wherein R_a is methyl or t-butyl.

4. The compound according to Claim 1 wherein the R_1 moiety is an optionally substituted alkoxy moiety.

5. The compound according to Claim 4 wherein the R_1 alkoxy is methoxy or 2-hydroxyethoxy.

6. The compound according to Claim 1 wherein m is 2.

7. The compound according to Claim 1 wherein R_3 is $S(O)_n R_6$.

8. The compound according to Claim 7 wherein R_6 is a heteroaryl which is an optionally substituted tetrazole, triazole, or oxadiazole.

9. The compound according to Claim 1 wherein R_3 is hydrogen.

10. The compound according to Claim 1 which is:
tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide
Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide
3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

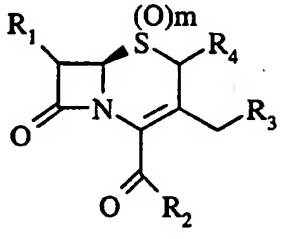
3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

11. A pharmaceutical composition comprising a compound according to Claim 1 and a pharmaceutically acceptable carrier or diluent.

12. A pharmaceutical composition comprising a compound according to Claim 10 and a pharmaceutically acceptable carrier or diluent.

13. A method of blocking excess or inappropriate apoptosis in a mammal in need of such treatment which method comprises administering to said mammal or human an effective amount of a compound of the formula:



(Ia)

wherein

R_1 is hydrogen, an optionally substituted alkoxy or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, $-OC(O)R_5$, $S(O)_n R_6$, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen, and that only one of R_3 and R_4 can be bromine;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;

R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;
n is 0, or an integer having a value of 1 or 2;
or a pharmaceutically acceptable salt thereof.

14. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs in Alzheimer disease.

15. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs in viral infections.

16. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs during infarction or reperfusion injury.

17. The method according to Claim 13 wherein the excessive or inappropriate apoptosis occurs during ischemia.

18. The method according to Claim 13 wherein the excessive or inappropriate apoptosis results in excessive bone loss.

19. The method according to Claim 13 wherein the excessive or inappropriate apoptosis results in the disease of osteoarthritis.

20. The method according to Claim 13 wherein the excessive or inappropriate apoptosis results in hepatocellular degeneration.

21. The method according to Claim 13 wherein the compound is:
tert-Butyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7R)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4-Dichlorobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
tert-Butyl (6R,7S)-3-acetoxymethyl-7-(2-hydroxyethoxy)-3-cephem-4-carboxylate-1,1-dioxide
Methyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
Benzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
3,4- and 2,3-Dimethylbenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide
4-Nitrobenzyl (6R,7S)-3-acetoxymethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl (1RS,6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate 1-oxide

3,4-Dichlorobenzyl-(6R,7R)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

4-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3-Iodobenzyl-(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3-Iodo-4-methylbenzyl -(6R,7S)-7-methoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-[2-hydroxyethoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-[n-butoxy]-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl -(6R,7S)-7-ethoxy-3-acetoxymethyl-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-bromomethyl-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

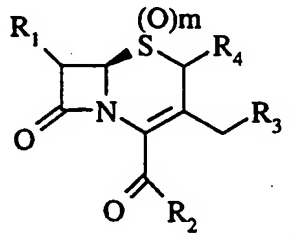
3,4-Dichlorobenzyl-(6R,7S)-3-phenylsulfonylmethyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[5-methyl-(1,3,4-oxadiazol)-2-thiomethyl]-7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1-methyltetrazole)-5-thio]methyl -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

3,4-Dichlorobenzyl-(6R,7S)-3-[(1,2,3-triazole)-4-thiomethyl] -7-methoxy-3-cephem-4-carboxylate-1,1-dioxide

22. A method for the treatment of diseases or disorders associated with excessive IL-1b convertase activity, in a mammal in need thereof, which method comprises administering to said mammal an effective amount of the formula:



(Ia)

wherein

R_1 is hydrogen, an optionally substituted alkoxy or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, -OC(O)R_5, $S(O)_n$ R_6, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen, and provided that only one of R_3 and R_4 can be bromine;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;

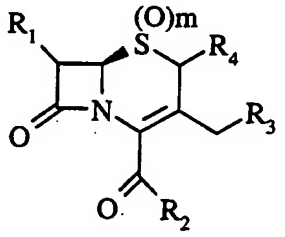
R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;

n is 0, or an integer having a value of 1 or 2;

or a pharmaceutically acceptable salt thereof.

23. A method of blocking or decresing the production of IL-1b and/or TNF, in a mammal in need of such treatment, which method comprises administering to said mammal an effective amount of a compound of the formula:



(Ia)

wherein

R_1 is hydrogen, an optionally substituted alkoxy or halogen;

R_2 is OR_a;

R_a is C_{1-4}alkyl, or optionally substituted aryl C_{1-4}alkyl;

R_3 is hydrogen, -OC(O)R_5, $S(O)_n$ R_6, or bromine; provided that when R_3 is hydrogen, R_4 is other than hydrogen, and that only one of R_3 and R_4 can be bromine;

R_4 is hydrogen;

R_5 is C_{1-6} alkyl, C_{3-7} cycloalkyl, optionally substituted aryl, optionally substituted arylalkyl;

R_6 is optionally substituted aryl, or optionally substituted heteroaryl;

m is an integer having a value of 1 or 2;

n is 0, or an integer having a value of 1 or 2;

or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13967

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07D 501/00; A61K31/545
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 540/215, 226, 229, 230;
514/204, 208, 209, 200

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN-CAS-on line
structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US, A,5,446,037 A(MAITI et al) 29 August 1995, column 3 and claim 1 when R4, here, is other than hydrogen.	1-23 1-23
X -P Y	Chemical Abstract, vol. 124, no.5. 29 January 1996(Columbus,OH,USA)page 1203,column 1,the abstract no.55675h. Alpengiani et al. WIPO document no. 94/28003, 08 December 1994.	1-23
X -- Y	Chemical Abstract, vol. 122,no.3.16 January 1995(Columbus OH. USA)page 905,column 1,the abstract no 31161n, Alpegiani et al., "Cephem Sulfones as Inactivators of Human Leukocyte Elastase", J. Med. Chem. (1994), vol. 37(23), pages 4003-19.	1-23 1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 12 DECEMBER 1996	Date of mailing of the international search report 03 FEB 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN M. FORD aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13967

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

540/215, 226, 229, 230;
514/204, 208, 209, 200